Heartworm disease (HWD) is an important disease of dogs and cats. It has been diagnosed throughout the United States, has a range of clinical presentations, and, in severe cases, can cause death. Management of HWD requires an understanding of the parasite's life cycle, clinical manifestations of the infection, preventive and treatment approaches, and knowledge of how to incorporate diagnostic tools to help identify infected animals. This technical bulletin reviews key details about infections, clinical disease, and considerations for heartworm testing. Additionally, there is a review of RIM™technology and how the WITNESS® HEARTWORM Antigen Test Kit works. Lastly, there is an overview of recent enhancements to the WITNESS® HEARTWORM Test kit and supporting study information demonstrating the impact of these changes.

Section 1: Heartworm Disease Overview
Section 2: WITNESS® HEARTWORM and RIM™
Section 3: WITNESS® HEARTWORM Supporting Data

The licensing studies for the WITNESS® HEARTWORM test submitted to the USDA Center for Veterinary Biologics demonstrate robust sensitivity, specificity, and product characteristics.
Heartworm infection (dirofilariasis) is caused by the filarial nematode *Dirofilaria immitis*, which is transmitted between animals via infected mosquitoes that deposit larval heartworms as they feed (Figure 1). Heartworm disease is reported worldwide, and has been diagnosed in all 50 states in the US. It is considered to be continuously present in the animal population of the contiguous 48 states, Hawaii, Puerto Rico, US Virgin Islands, and Guam. Heartworm infections have been reported in domestic dogs, domestic cats, wolves, foxes, coyotes, ferrets, muskrats, sea lions, nondomestic cats, coatimundi, and, rarely, humans.2–3

**Canine Heartworm Disease**

Dogs are generally considered the definitive host for heartworms (Table 1). While taking a blood meal, an infected mosquito deposits infective third stage larvae (L3) after feeding. The larvae then infect the dog through the mosquito bite wound in the dog’s skin. For the next 70 to 120 days, larvae develop while migrating through the subcutaneous and muscle tissues, molt twice to reach the final adult stage, and eventually penetrate smaller blood vessels as they move toward the heart and adjacent large blood vessels.1 As early as 6 months after infection, but more typically within 7 to 9 months post infection, mating occurs, and fertilized females begin releasing microfilariae into the blood stream of the host, resulting in a patent infection.1

In dogs, clinical signs of HWD vary according to the severity and duration of infection. The presence of adult worms causes damage primarily to the pulmonary arteries and lungs and secondarily to the heart. Early in any infection, many dogs appear clinically normal. With a mild to moderate infection, dogs may cough, show exercise intolerance, and have abnormal lung sounds. With a severe infection, dogs may show additional signs that can include enlargement of the liver, temporary loss of consciousness, fluid accumulation in the abdomen, abnormal heart sounds, and death.4

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Figure 1 – Heartworm life cycle in dogs and cats. Adapted from the American Heartworm Society (www.heartwormsociety.org/pet-owner-resources/heartworm.html. Accessed December 1, 2012.).
In most cases, dogs infected with adult heartworms can be successfully treated by targeting the adult worms and microfilariae; however, special consideration must be given to the potential harmful effects of the drugs administered and the sequelae of dead and dying heartworms and microfilariae. Typically, treatment of infected dogs showing no or only mild signs has a high success rate. Dogs with more severe heartworm disease can also often be treated successfully, but the incidence of post-treatment complications and mortality is greater.

**Feline Heartworm Disease**

Cats are susceptible hosts for heartworm infections (Table 1). *Dirofilaria immitis* infects cats the same way as dogs, although the time it takes to develop into adult worms is longer, and worms are less likely to reach full maturity. Clinical signs can occur as a consequence to damage caused by dying immature worms. This has been identified as a condition known as Heartworm Associated Respiratory Disease (HARD).

For cats with adult heartworms, many are asymptomatic or have subclinical disease, but the death of mature heartworms can result in collapse, difficult breathing, convulsions, vomiting, diarrhea, blindness, and, in rare cases, sudden death. Chronic signs of a heartworm infection include coughing, vomiting, difficult breathing, and lethargy.

Currently, no products are approved in the US for the treatment of feline heartworm infections. Once diagnosed, infected cats should be monitored for complications and provided with supportive care as needed. Some cats with mild signs may resolve the infection spontaneously with little to no intervention. Surgical extraction of adult worms has been performed in some instances with heavily infected cats and those cats with obstruction of blood flow returning to the heart and liver (vena caval syndrome).

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**Table 1. COMPARISON OF CANINE AND FELINE HEARTWORM INFECTIONS**

Adapted from Blagburn BL and Dillon AR.

<table>
<thead>
<tr>
<th>DOG</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dogs are definitive hosts. Most (if not all) infected dogs will develop adult heartworms.</td>
<td>• Cats are atypical hosts. Many fewer cats than dogs develop adult heartworms when exposed to infected mosquitoes.</td>
</tr>
<tr>
<td>• Most immature heartworms in the lungs of dogs mature to adult heartworms.</td>
<td>• It is thought that many immature heartworms in the lungs of cats fail to mature to adult heartworms.</td>
</tr>
<tr>
<td>• Infected dogs often have &gt; 30 adult heartworms.</td>
<td>• Infected cats usually harbor fewer than 6 adult heartworms (commonly 1-3 worms).</td>
</tr>
<tr>
<td>• Most dogs that do not receive adulticide or preventive medications develop circulating microfilariae. Microfilaremia can persist for many months.</td>
<td>• Only 50% of cats develop circulating microfilariae. Microfilaremia persists for just one to two months.</td>
</tr>
<tr>
<td>• Canine heartworm disease, particularly severe forms, affects both the heart and lungs.</td>
<td>• Feline heartworm disease affects the lungs much more often than the heart.</td>
</tr>
<tr>
<td>• In dogs there is an approved adulticide.</td>
<td>• Generally, only symptomatic treatment is practiced in cats. Approved adulticides are lacking; use of canine adulticides is not recommended.</td>
</tr>
</tbody>
</table>
Heartworm Prevention

Because of costs associated with managing or treating heartworm disease, potential complications associated with treatment, and the lack of an approved treatment option for cats, disease prevention is regarded as the preferred method of heartworm disease control in both dogs and cats. Several FDA-approved products are available for preventing migrating larvae from developing into mature heartworms in both species. When administered in accordance with product label instructions, these products are highly effective in preventing the development of heartworm disease.4,6

Heartworm Disease Diagnosis

The diagnosis of heartworm disease may be made after implementing and analyzing the results of one or more of the following diagnostic tools: a complete clinical history; physical examination findings; testing for heartworm antigen, antibody, or microfilariae; thoracic radiographs; and echocardiography. Table 2 summarizes some important considerations for diagnosing heartworm infections in dogs and cats.

Diagnosing Heartworm Infection in Dogs

Heartworm antigen and microfilariae testing are routinely used to diagnose or confirm heartworm infections in dogs. Heartworm antigenemia usually can be detected in dogs starting 5 to 6 months post infection, but may be delayed in dogs receiving heartworm preventives.1 Antigen testing is routinely used to screen dogs prior to starting preventive medications, as well as to diagnose dogs

For up-to-date recommendations on heartworm disease, prevention, and treatment, refer to the American Heartworm Society’s Current Canine and Feline Guidelines at:
Guidelines


Canine/feline guidelines in English, French, Spanish, and Italian are available at this site.

Table 2. HEARTWORM DIAGNOSTIC PROCEDURES AND TESTS IN THE DOG AND CAT1,5,11

<table>
<thead>
<tr>
<th>Diagnostic Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DOG</strong></td>
</tr>
<tr>
<td>• Antigen testing is routinely recommended for screening dogs on heartworm preventive.</td>
</tr>
<tr>
<td>• Confirmation testing should be considered for positive dogs with low exposure, or that are asymptomatic.</td>
</tr>
<tr>
<td>• Depending on the clinical status of each patient prior to treatment, additional diagnostic testing may be indicated to complement the dog’s history, physical examination, and heartworm test results.</td>
</tr>
</tbody>
</table>

| **CAT**                  |
| • Positive antigen test confirms an infection with adult heartworms. |
| • Positive antibody test suggests infection with heartworm larvae, increases index of suspicion of an adult heartworm infection, confirms that the cat is at risk, but does not confirm the presence of adult heartworms. |
| • A comprehensive approach to diagnosis may be required to rule in, or rule out, heartworm infection in cats, including serologic testing (antigen/antibody), thoracic radiographs, echocardiography, or, in case of death, necropsy. |
suspected of being infected with adult *D. immitis*.

Microfilaremia in dogs typically can be detected by 6 to 7 months after infection.\(^1\) Despite rare reports of dogs with circulating microfilariae and no detectable adult heartworm antigen (< 1%),\(^2,7\) testing for microfilariae is usually regarded as complementary to antigen testing because in heartworm endemic areas 5% to 67% of infected dogs harbor both male and female heartworms but no microfilariae.\(^2,8\) The percentage of occult infections (adult worms present, but no circulating microfilariae) is thought to be even higher in areas where dogs routinely receive some degree of heartworm prophylaxis, because of the microfilaricidal effects of the preventive medications.\(^1,2\)

Although microfilariae testing is generally not used for primary heartworm screening, testing for microfilariae still plays an important role in validating serologic results in dogs when microfilariae are present. Identifying a dog as a reservoir of infection can alert the veterinarian to the potential for a severe reaction if a microfilaricide were to be administered to a dog with a high microfilarial count.\(^1\)

Prior to initiating adulticide treatment, especially for asymptomatic dogs at minimal risk for exposure, the American Heartworm Society recommends confirming all heartworm positive antigen tests.\(^1\) Depending on the clinical status of each patient, additional diagnostic testing, may be indicated to complement a dog’s history, physical examination, and heartworm antigen and microfilariae test results.\(^1\)

### Diagnosing Heartworm Infection in Cats

In cats, a more comprehensive approach to heartworm testing may be required because:

- Infected cats have both lower worm burdens and lower antigen levels
- Immature/larval infections have increased importance in the species\(^5,9-11\)

Both antigen and antibody testing are routinely used to evaluate cats for heartworm infections. A positive antigen test confirms the presence of an infection with adult worms. Adult heartworm antigen in circulating blood can be identified in cats at approximately 5½ to 8 months after infection.\(^11\) Antibody testing can be used to confirm heartworm exposure and development of heartworm larvae as early as 8 weeks after infection.\(^11\) The positive result of a heartworm antibody test could signal that a cat has an adult infection, or it could be at risk for HARD, or future infection, but it does not confirm the presence of adult worms.\(^10,12\) Heartworm antigen and antibody tests are useful tools that can complement one another and assist in making an appropriate diagnostic decision.\(^11\) Testing for both antibody and antigen improves sensitivity compared with running either test alone.\(^9,13,14\) Microfilariae are rarely observed in infected cats, and, when they do develop, generally are detectable only for a limited time; as such, this limits the utility of microfilaria testing.\(^11,15,16\)

In clinical situations where there is an antemortem suspicion of heartworm infection, a comprehensive approach to diagnosing heartworm disease could include: antigen testing, antibody testing, thoracic radiographs, and echocardiography.\(^9-12\) In cases of unexplained death, necropsy results may be useful in identifying some infected cats.
Heartworm Antigen Testing Regularly Used in Veterinary Practice

At the foundation of most heartworm infection diagnoses is a heartworm antigen test. Readily available, accurate, easy to run, and affordable, commercial point-of-care and laboratory heartworm antigen tests are widely used for both dogs and cats. Commercially available point-of-care and reference laboratory heartworm antigen test kits detect antigen that is released primarily by adult female heartworms, and the amount of antigen in circulation is generally related to the number, age, and health of the worms. Most heartworm antigen tests accurately detect infections with at least a few mature female heartworms. Differences between tests include run time, number of steps involved, sample requirements, storage requirements, and approved species for use.

The first WITNESS® brand point of care diagnostic line utilizing Rapid Immuno Migration, or RIM™technology was introduced in Europe in 1995. RIM is a test format that uses tagged colloidal gold particles as a color signal rather than an enzyme-catalyzed color change reaction as in ELISA to visualize the presence of heartworm antigen (Figure 2).

Colloidal gold is used as the signal because its inherent stability allows the WITNESS® HEARTWORM Antigen Test Kit to be stored without refrigeration for the duration of the test kit’s shelf life. The WITNESS® HEARTWORM Test Kit provides patient-side heartworm results in approximately 10 minutes.

WITNESS® HEARTWORM: How It Works

- One drop, 0.05 mL (50 µL) of sample (Table 3) is added directly to the sample well from the provided pipette (Figure 3). After the sample is absorbed, 2 drops of buffer are added to the same sample well.
- The sample and buffer flow through the sample pad where blood cells and cellular debris are filtered, allowing the test sample to flow onto and across the conjugate pad and nitrocellulose membrane test strip.

Table 3. APPROPRIATE SAMPLE TYPES FOR THE WITNESS® HEARTWORM CANINE AND FELINE HEARTWORM ANTIGEN TEST

1. Anticoagulated whole blood (EDTA/heparin)
2. Serum
3. Plasma

- Antigen specific \(D. immitis\) antibody-colloidal gold complex (conjugate) binds any heartworm antigen within
the sample. Antigen bound complexes and control line targeted colloidal gold antibodies migrate laterally across the strip.

- A second antibody specific for *D. immitis* antigen is immobilized on a “result (test) line” of the nitrocellulose membrane. It binds heartworm antigen as it flows across the test strip causing a pink to purple color line to develop on the result (test) line.

- Control line targeted colloidal gold-tagged antibody continues to flow across the membrane towards the absorption (wicking) pad and is bound at the control line. A visible control line verifies that the test is working properly.

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**Figure 3 – WITNESS® HEARTWORM Antigen Test Kit for dogs and cats.**

- **a)** Sample well, Result window, Result line, Control line, Housing, Sample pad, Conjugate pad, Nitrocellulose membrane, Absorption (wicking) pad.

- **b)** To initiate the test, one drop of sample and 2 drops of buffer are added to the sample well.

- **c)** The sample/buffer and conjugate migrate across the nitrocellulose strip.

- **d)** Canine heartworm antigen bound conjugate is captured at the result line, and control line targeted conjugate is captured at the control line.
The WITNESS® HEARTWORM Antigen Test Kit is an improved version of the WITNESS® HW and WITNESS® DIROFILARIA tests sold in different markets globally. The first WITNESS® test for the detection of heartworm antigen was introduced in 1996 in France, and 1997 in the U.S. In 2013, enhancements affecting multiple areas of the manufacturing process and test antigens were incorporated into the test with the intention of helping improve test sensitivity and product performance. Later, upon discovery of an interfering substance leading to false positive test results in a small population of dogs, additional modifications were made to the test to improve its specificity. As a result of the various changes and the beginning of the process of unifying the different global brands, in 2015 Zoetis updated the brand name to WITNESS® HEARTWORM.

The cumulative effects of the improvements were demonstrated and the sensitivity and specificity of the test were confirmed in a submission to the USDA Center for Veterinary Biologics. Results from more than 700 samples from a variety of different patient populations including shelter dogs, veterinary clinic sourced samples, and purpose-bred laboratory dogs were submitted for evaluation (Table 4). Key results of the studies supporting the changes are presented in Tables 4 and 5.22,23

### Summary of changes to WITNESS® Heartworm Antigen Test Kit done in 2013 and 2015

1. Enhancements to the biologics, including test antibodies and the colloidal gold signal
2. Enhancements to the buffer and the sample pad
3. Enhancements to improve the signal consistency at the control line
4. Enhancements to neutralize a substance found in a sub-population of animals that previously yielded false positive test results

### 1. SENSITIVITY and SPECIFICITY in serum collected from shelter dogs (n = 285). This sample set was from dogs that were deemed sick or behaviorally unadoptable from various shelters in Florida. The sample set included a large number of positive and negative dogs.

### 2. SPECIFICITY in plasma samples collected from dogs presenting to veterinary hospitals (n = 342). These samples were collected from dogs presenting to three veterinary hospitals in Georgia, New York, or Michigan.

### 3. SPECIFICITY in whole blood and plasma samples collected from purpose-bred laboratory dogs (n = 149). These samples were from low-risk purpose-bred laboratory dogs.
In a separate evaluation, the 285 serum samples collected from shelter dogs discussed in Table 4 were also tested against SNAP® 4Dx (IDEXX Laboratories) with DiroCHEK® (Zoetis) as the reference standard. Test results are presented in Table 5.

The results of additional specificity studies are presented in the box below:

### Table 4: WITNESS® HEARTWORM VS. DIROCHEK® IN SHELTER DOGS

<table>
<thead>
<tr>
<th>WITNESS® HEARTWORM Antigen Test</th>
<th>DiroCHEK® Heartworm Antigen Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>97.8% (95% CI: 94.1%–99.4%)</td>
<td>100% (95% CI: 98.4%–100%)</td>
</tr>
</tbody>
</table>

### Table 5: COMPARATIVE STUDY RESULTS OF WITNESS® HEARTWORM AND SNAP®4Dx VS. DIROCHEK® AS THE REFERENCE STANDARD IN SERUM SAMPLES COLLECTED FROM 285 SHELTER DOGS

<table>
<thead>
<tr>
<th>Test</th>
<th>WITNESS® HEARTWORM</th>
<th>SNAP® 4Dx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>97.7%</td>
<td>94.7%</td>
</tr>
<tr>
<td>95% CI</td>
<td>94.1%–99.4%</td>
<td>89.9%–97.6%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.3%</td>
<td>100%</td>
</tr>
<tr>
<td>95% CI</td>
<td>97.0%–99.9%</td>
<td>98.4%–100%</td>
</tr>
</tbody>
</table>

Additional Specificity Studies

(1) In an evaluation of dogs from three veterinary hospitals from three states (Georgia, Michigan, and New York), 341/342 DiroCHEK® negative samples were negative on WITNESS® HEARTWORM (99.7% specificity, 95% CI: 95.5%–100%). Data on file, Study Report No. D860Z-US-15-018, Zoetis Inc.

(2) In an evaluation of purpose-bred laboratory dogs, 149/149 samples that were negative on the DiroCHEK® test were also negative on the WITNESS® HEARTWORM test (100% specificity, 95% CI: 98.3%–100%). Data on file, Study Report No. D860Z-US-15-018, Zoetis Inc.

A common use of heartworm testing is in dogs prior to continuing use of heartworm preventive products. In general, this population is at low risk based on the assumption the products were given as directed. Test specificity, or the ability to correctly identify uninfected dogs as negative, is very important in this population.
CONCLUSIONS

Despite the availability of safe and highly effective medications that prevent the maturation of infective larvae into adult heartworms, heartworm infection remains a significant threat to the health and well-being of the dog and cat populations of the US. Key to successful medical management of heartworm disease is early and accurate identification of the infected animal.

Various diagnostic tools and procedures are sometimes necessary to diagnose or confirm a heartworm infection. However, antigen tests are frequently at the foundation of most diagnoses in dogs as they are the most effective method for identifying both patent and occult heartworm infections by detecting circulating antigens specific to D. immitis. They are also useful in cats, and are often used in combination with other diagnostic tools, including antibody detection tests, radiography, and echocardiography.

The WITNESS® HEARTWORM Antigen Test Kit for dogs and cats uses Rapid Immuno Migration – RIM™ technology to visualize the presence of heartworm antigen in three different sample types. The entire test can be completed pet-side in approximately 10 minutes. Modifications made to its manufacturing processes and test components since the original test was introduced have improved its sensitivity and specificity, as well as product performance.

Overall, the WITNESS® HEARTWORM Test Kit is an excellent choice for diagnosing or confirming a heartworm infection in dogs and cats.
References


